Neurosci Bull June 1, 2009, 25(3): 161-164. http://www.neurosci.cn
DOI: 10.1007/s12264-009-0120-3

·Minireview·

P2Y6 receptor and immunoinflammation

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Abstract: The immunocytes microglia in the central nervous system (CNS) were reported to play a crucial role in neurodegeneration. As a member of P2 receptors family, purinoceptor P2Y6 has attracted much attention recently. Previous studies showed that purinoceptor P2Y6 mainly contributed to microglia activation and their later phagocytosis in CNS, while in immune system, it participated in the secretion of interleukin (IL)-8 from monocytes and macrocytes. So there raises a question: whether purinoceptor P2Y6 also takes part in neuroinflammation? Thus, this review mainly concerns about the properties and roles of purinoceptor P2Y6, including (1) structure of purinoceptor P2Y6; (2) distribution and properties of purinoceptor P2Y6; (3) relationships between purinoceptor P2Y6 and microglia; (4) relationships between purinoceptor P2Y6 and immunoinflammation. It's proposed that purinoceptor P2Y6 may play a role in neuroinflammation in CNS, although further research is still required.

Key words: purinoceptor P2Y6; microglia; inflammation; immunology; neurodegeneration

1 Introduction

Extracellular nucleotides are ubiquitous molecules that initiate and regulate a myriad of physiological effects via membrane-bound purinoceptors. Nucleotide receptors are normally divided into two categories: P1 receptors that bind to adenosine in a concentration-dependent way and have diverse biological functions through G-protein linked second-messenger systems, and P2 purinoceptors that are further divided into two families: ionotropic receptors (P2X) family and metabotropic receptors (P2Y) family. P2X receptors (7 types: P2X1–P2X7) contain intrinsic pores that are opened following ATP binding[1]. P2Y receptors (8 types: P2Y1, 2, 4, 6, 11, 12, 13 and 14) are activated by nucleotides and linked to intracellular second-messenger systems through heteromeric G-proteins. Nucleotides are released or leaked from non-excitable cells as well as neurons under physiological or pathophysiological conditions[2]. Under normal conditions, some P2Y receptors, including P2Y2, P2Y4 and P2Y6 receptors, participate actively in regulating the secretions of Cl^-, Na^+ and K^+ and in maintaining the Ca^{2+} concentration[3]. However, under inflammatory conditions, activations of P2Y receptors can stimulate cell proliferation and play a role in immune cell recruitment, proliferation and differentiation, thus enhance the expressions and secretions of cytokines and proinflammatory molecules, and increase the expressions of cell adhesion molecules and promote the cell migration[4-7]. To learn more about P2Y receptors, we choose purinoceptor P2Y6 as an example and discuss recent research development on P2Y6.

2 Structure of purinoceptor P2Y6

Like other receptors of the P2Y family, purinoceptor P2Y6 has seven transmembrane domains (Fig.1). The N-terminal region contains a single potential asparagine-linked glycosylation

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Article ID: 1673-7067(2009)03-0161-04
CLC number: Q421; R749
Document code: A
Received date: 2009-01-20

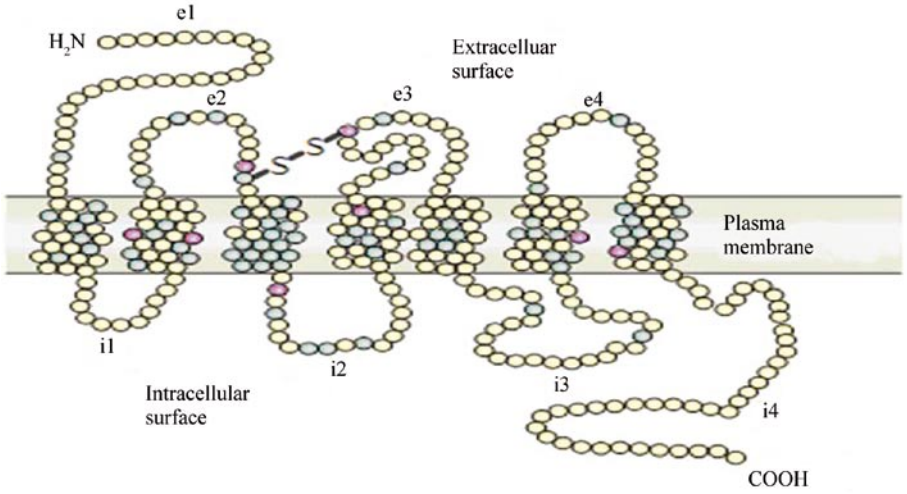

Figure. 1 The P2Y receptors family belong to G-protein-coupled receptors (adapted from Fields RD, Nat Rev Neurosci 2006)[12].

site, and the third intracellular loop and cytoplasmic C-terminal have two recognition sites (Ser-235 and Thr-320) for phosphorylation by protein kinase C[8,9,13]. The human P2Y6 receptor gene is localized in chromosome 11q13.3-13.5[10]. cDNA cloning of P2Y6 receptor is identified in three cDNA isoforms, among which two isoforms have identical contiguous open reading frames (ORFs) but differ in their 5' untranslated regions (5' UTRs), originating probably from alternative splicing, and the third isoform represents a pseudogene[11].

3 Distribution and properties of purinoceptor P2Y6

P2Y6 receptor is widely distributed in various tissues, including placenta, spleen, thymus, small intestine, blood, heart, blood vessels and brain[13,14]. On the cellular level, P2Y6 receptor is expressed in many kinds of cells, including intestinal epithelial cells, T cells (affected T cells), monocytes, microglia, vascular endothelia cells, cardiomyocytes, smooth muscle cells[15,16] and neurons in the guinea pig enteric nervous system[17]. Its mRNA expression has also been detected in motor neurons[18] and spinal sensory neurons. Diphosphonucleotides such as UDP and UTP are ligands of P2Y6 receptor, and they are ordered according to the disparity in potency as follows: UDP > TDP > IDP > GDP > ADP >> CDP [19]. As a ligand of P2Y6 receptor, UDP is approximately 100-fold more potent than UTP[14,20], whereas ADP, ATP and their 2-methylthio derivatives are almost inactive. The specific antagonist for P2 receptors is MRS2578. Other three non-specific antagonists were ordered according to their activities as in the follows: reactive blue> pyridoxal-phosphate-6-azophenyl-2', 4'- disulphonicacid (PPADS) > suramin.

4 P2Y6 receptor and microglia

Expressing many types of P2 purinoceptors, microglia are known as resident macrophages in the central nervous system (CNS). ATP and other nucleotides work as warning molecules especially through activating microglia under pathophysiological conditions. Microglia may play a key role in chemotaxis, phagocytosis and neuroinflammation through nucleotide-evoked activations of P2X4, P2Y12 and P2Y6 receptors. Microglia are well known as sensors of most of the brain-damaging events and are very crucial in the progress of many neurodegenerative diseases, such as Parkinson disease (PD), amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD). Microglia will be activated as a response to injury, resulting in their interactions with immune cells. Then activated microglia migrate to the sites of injury, releasing proinflammatory mediators and finally engulfing the damaged cells and cell debris, the process of which is known as phagocytosis. Koizumi and his colleagues reported that it was the P2Y6 receptor that mediated the phagocytosis of microglia, and they also proposed that UTP released from damaged cells might trigger phagocytosis

through P2Y6 receptor[21]. In other words, the investigations in the role of P2Y6 receptor in mediating microglia phagocytosis could help develop therapeutic agents to interfere with microglia activation in diseases.

5 P2Y6 receptor and immunoinflammation

The presence of P2Y6 transcripts in human spleen, thymus and blood leukocytes suggests a possible role of P2Y6 receptor in the immune system. As a selective P2Y6 agonist, UDP can stimulate the release of interleukin (IL)-8 from human THP-1 monocytes and intestinal epithelial cells, whereas other nucleotides are relatively inactive[22,23]. Other inflammatory stimuli, such as tumor necrosis factor (TNF-α), interferon (IFN)-γ and LPS can also stimulate IL-8 release from monocytes, intestinal epithelial cells and monocyte-derived dendritic cells via P2Y6 receptor through an ERK1/2-dependent way[22-25]. Extracellular nucleotides can also regulate release of chemokine (C-C motif) ligand 20 (CCL20) from human primary airway epithelial cells, monocytes and monocyte-derived dendritic cells through P2Y6 receptor[25]. These findings indicate a novel role of P2Y6 receptor in innate immune defenses. P2Y6 knockout mice are viable and their growth or fertility is indistinguishable from that of the wild-type mice. However, P2Y6 knockout macrophages fail to show enhanced release of either interleukin (IL)-6 or macrophage-inflammatory protein 2 (MIP-2) in response to LPS stimulation, while they exhibit enhanced release of TNF-α. What's more, the endothelial dependent relaxation of the aorta by UDP, and the contractile effect of UDP on the aorta observed in the block of endothelial nitric-oxide synthase, were also absent in P2Y6-null mice[26].

6 Conclusion

From these observations, we conclude that P2Y6 receptor plays an important role in immunoinflammation in peripheral system, mainly by modulating the production and secretion of IL-8 in monocytes and macrophages. We also propose that P2Y6 receptor may be involved in neuroinflammation in neurodegenerative diseases. The role of P2Y6 receptor in CNS especially in microglia-associated processes still needs further investigation. Uracil nucleotide-sensitive P2Y receptor subtypes may become future targets for treatment of neurodegenerative diseases, vascular diseases and inflammatory diseases.

Acknowledgement: This work was supported by the Key Scientific Research Innovation Program of Shanghai Municipal Education Commission, China (No.082260).

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P2Y6 受体与免疫炎症

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摘要：小胶质细胞是中枢神经系统中的一种免疫活性细胞，在神经退行性疾病的发生发展中起着重要作用，近年已经引起基础研究者的高度重视。嘌呤能受体P2Y6是P2受体家族中的一员，近年来也引起了科学界的关注。有研究表明，P2Y6受体在中枢神经系统中介导小胶质细胞的活化及其吞噬作用，在免疫系统中参与单核巨噬细胞分泌释放细胞因子IL-8。在中枢神经系统中P2Y6受体是否也参与了炎症因子的产生呢？本文就以下四个方面对P2Y6受体做一综述:(1)P2Y6受体的结构; (2)P2Y6受体的分布及特性; (3)P2Y6受体与小胶质细胞的关系; (4)P2Y6受体与免疫炎症的关系。推测P2Y6受体可能在中枢神经系统的免疫炎症反应中起重要作用，仍需进一步的研究去证实。

关键词：P2Y6受体；小胶质细胞；炎症；免疫；神经退行性疾病